January 31, 1889.

Professor G. G. STOKES, D.C.L., President, in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Paper was read:—

I. "On *Isoëtes lacustris*, Linn." By J. BRETLAND FARMER, B.A., F.L.S. Communicated by Professor S. H. VINES, F.R.S. Received January 22, 1889.

The genus *Isoëtes* has been an object of interest to botanists ever since Hofmeister's brilliant researches on the vascular cryptogams, but the accounts given by the different observers on the development and organogeny of the sporophyte are so conflicting, and moreover our knowledge of the sexual generation is so limited, that a renewed investigation of the whole subject seems desirable. In the present communication I propose to summarise, as briefly as possible, the more important of my own observations on one species, *I. lacustris*, to which plant my attention has been directed for some time past. I intend to deal here only with the germination of the macrospore, and to reserve details of minor significance, as well as all account of the sporophyte, for treatment in a future paper, as this part of the subject requires critical discussion.

The shape of each macrospore is, as is well known, that of a tetrahedron with somewhat rounded sides, and the protoplasmic contents are enclosed in a number of coats which, in mature specimens, are differentiated into six layers. Peripherally is the episporium, a colourless, glassy, and brittle layer, whose surface is beset with numerous irregular prominences. The episporium, which is derived from the epiplasm of the sporangium, stains with hæmatoxylin, though only to a slight extent. Within this outer layer is the exosporium, consisting of three brown cuticularised layers, but of which the two outer ones are frequently not easily distinguishable as separate coats. The two innermost membranes of all, are cellulose in character, and form the endosporium.

The protoplasm which is contained in the spore includes a large quantity of reserve material, consisting of starch and oil, the latter being, however, eliminated during the process of soaking in turpentine, to which the spores are subjected previously to their being

embedded in paraffin. A number of sections through each spore were obtained by means of the Cambridge rocking microtome, and were arranged in series, thus permitting of an examination of the internal structure of the spores. The protoplasm, which is remarkably granular, is of a spongy texture (probably due to the extraction of the oil), and contains a nucleus of very large size, in which bodies resembling nucleoli were in some cases detected. The nucleus is sharply marked off from the cytoplasm by a membrane, but of course it must be borne in mind that this feature may be caused in part by the methods used in embedding. When spores are examined in this stage, the protoplasm stains but slightly with hæmatoxylin, and the tint is inclined to red, and even the nucleus is not deeply coloured. In somewhat older spores, at the period immediately preceding germination, the whole protoplasm stains far more readily and deeply in a given time, but a nucleus is no longer differentiated by the hæmatoxylin, and the colour now produced is of a deep blue. As I have frequently had spores of different ages on the same slide, all of which were subjected to exactly similar treatment, this difference in colour may probably be taken to indicate an actual diffusion of the substance of the nucleus through the cytoplasm, since the change is always confined to spores in the condition referred to.

This view receives some confirmation from the circumstances attending the formation of the prothallium, now to be described. The first indication of cell-division occurs in a somewhat peculiar manner, but its significance is rendered clear by what takes place subsequently. Before entering upon a description of what actually happens, it may be well, in order to avoid possible misconstruction, to state expressly the opinion that the characters presented are made visible only by the action of the means necessarily employed in embedding, but this does not vitiate the conclusion that they may be taken as indications of internal changes which actually occur in the protoplasm. In spores in which cell formation is about to commence, the deeply stained protoplasm is seen to be traversed by a few "cracks," which divide the contents of the spore into large isolated masses. At this period there is nothing to point to the existence of a membrane, except the granular structure which is apparent on the surface of the cracks, but at a subsequent stage in the development, one of the surfaces is seen to be bounded by a membrane of extreme tenuity. When first formed it can only be distinguished in favourable places, but it rapidly grows in thickness, and forms a limiting surface between the two protoplasmic masses. From the mode of its formation it can hardly arise otherwise than by the conversion of a layer already present in the protoplasm directly into cellulose, and it appears to be the presence of this substance arranged in a definite plate-like manner which determines the splitting referred to. The first membrane cuts

the spore into an apical and a basal portion, and while the latter for some time undergoes no further change, the apical cell is divided very rapidly into a number of cells, whose arrangement can still be followed even in quite old prothallia. When the first primary cells are formed, the nuclei are again distinguishable on staining with hæmatoxylin, but they are of exceedingly small dimensions, and with this change the staining capacity of the protoplasm becomes less marked. Divisions in all planes proceed very rapidly in the upper (apical) portion of the prothallium, and the rudiments of the archegonia are laid down much as in the Marattiaceæ. Periclinal division of single superficial cells into two takes place, the upper of which gives rise to the neck, and by repeated division forms four stories, each story being again divided crosswise into four cells arranged as quadrants of a cylinder. The lower cells form the central series, in which a neck canal cell is cut off, and then a ventral canal cell, from the oosphere. The canal cells then thrust themselves between the neck cells, and cause a distortion in the two lower stories, which may be so great as even to render them difficult of recognition.

Whilst these changes have been taking place in the upper (apical) of the two primary cells, the lower (basal) one is dividing, but comparatively slowly, and it is easily distinguishable in that the cells arising from it remain of a large size as compared with those formed in the upper part of the prothallium. In spite of repeated search through a great number of preparations, it has not been found possible hitherto to arrive at a satisfactory conclusion as to the mode of cell-division which prevails in the secondary stage, for no karyokinetic figures could be detected; nevertheless, it is highly probable that the process does not differ in any important respect from that exhibited by other plants, and the arrangement of the nuclei about the walls of recently formed cells makes this supposition almost a certainty.

I have purposely omitted any reference to the researches of other observers in the present paper, and it was not my object to attempt a complete account of my own work, which is still in progress, but the results detailed above appeared of sufficient interest to justify the appearance of this note.